VIMSS: Large-Scale Biomass Production of Obligate Anaerobes for Simultaneous Transcriptomics, Proteomics, Metabolomics, and Lipidomics Analysis

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RESEARCH OBJECTIVES

The Virtual Institute for Microbial Stress and Survival (VIMSS) at Lawrence Berkeley National Laboratory (Berkeley Lab) seeks to identify stress-response pathways of *Desulfovibrio vulgaris* induced by various environmental factors, by combining multiple simultaneous analyses in an effort to conceptualize these pathways. The Applied Environmental Microbiology core at Berkeley Lab is responsible for producing large quantities of *D. vulgaris* biomass for different research laboratories, to accomodate simultaneous analyses on cells with the same growth condition and stress level. Biomass production at Berkeley Lab involves the production of nonstressed and stressed *D. vulgaris* culture at various time points during the growth phase, which is a four-month process with two major stages. During Stage 1, Berkeley Lab determines the stressor and the dosage, as well as designing and carrying out a multi-time point experiment for Oak Ridge National Laboratory's transcriptome analysis. During Stage 2, Berkeley Lab will produce 12 to 30 liters of biomass for all the VIMSS laboratories, based on the results of transcriptomics. Berkeley Lab is also responsible for all QA/QC verifications, all sample shipments, the uploading of data, and analysis for all experiments.

APPROACH

To rapidly determine induced-stress-response pathways in anaerobic microorganisms, we need to produce biomass for simultaneous analyses, using the latest techniques in transcriptomics, proteomics, metabolomics, and lipidomics. To accomplish this, batch cultures of 30 liters of stressed versus nonstressed *D. vulgaris*, as biological replicates in triplicates, are needed to ensure that all the analyses will be performed on cells of the same condition. Various technical improvements and adaptations were made for the large-scale production and distribution of biomass exposed to a variety of stressors, such as oxygen, salt, nitrate, nitrite, and temperature. Because of the rapidly changing nature of DNA and the short half-life of mRNA, *D. vulgaris* cultures needed to be immediately cooled to 5°C during biomass sampling. As a result, a fast sample-cooling system was developed to chill biomass from 30°C to 5°C in less than 30 seconds. Because of the concomitant analysis by several laboratories, rigorous quality control measures were used to ensure the quality and sterility of biomass from each time point in a production run (e.g., direct cell counts, optical density, pH, plate streaks, phospholipid fatty acid [PLFA] analysis, and protein assays). In addition, advanced Fourier Transform Infrared (FTIR) spectromicroscopy profiling was used to study gross bimolecular changes and to determine optimal sampling times. QA/QC procedures were developed and documented to track every step in production, from experiment inception to final analyses, including all chemicals, procedures, and technicians. Data are immediately uploaded to a database shared by all investigators (http://vimss.lbl.gov)

ACCOMPLISHMENTS

Over a 20-month period from September 2003 to May 2005, the Applied Environmental Microbiology core at Berkeley Lab conducted over 40 large-scale *D. vulgaris* biomass production experiments and countless small-scale experiments. During this time, the group developed various techniques and made numerous improvements to VIMSS biomass production, such as in media composition, anaerobic sampling, and biomass harvesting. Direct filtration and tangential flow filtration were studied as viable cell harvesting alternatives to centrifugation. However, studies showed that centrifuging *D. vulgaris* cells was the best option in terms of time, cost, efficiency, and quality control. The consistency of growth determined by the comparison data allows a set biomass production schedule and sampling time, which minimize variability between experiments.

SIGNIFICANCE OF FINDINGS

- Biomass production of batch cultures in biological replicates demonstrated a reliable and carefully controlled method to inoculate, grow, stress, and sample *D. vulgaris* cultures.
- QA/QC verifications at every stage of biomass production insure maximum reproducibility between biomass production experiments.
- Centrifugation and the fast chilling system appropriately prepared replicate samples simultaneously for transcriptomics, proteomics, metabolomics, and lipidomics processing.
- The large-scale biomass production of *Desulfovibrio vulgaris* for stress response studies can be used as a model for the large-scale production of other obligate anaerobes in the future.

RELATED WEB SITE

http://vimss.lbl.gov

ACKNOWLEDGMENTS

This work was part of the Virtual Institute for Microbial Stress and Survival supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics Program: Genomes To Life (GTL) through Contract No. DE-AC03-76SF00098 between Berkeley Lab and the U. S. Department of Energy.